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The role of hybridization in the karyotype evolution of deer (*Cervidae; Artiodactyla; Mammalia*)

S. Herzog[1] **and R. Harrington**[2]

[1] University of Göttingen, Department of Forest Genetics and Forest Plant Breeding, Büsgenweg 2, D-W-3400 Göttingen, FRG
[2] Mammal Conservation Research Section, Wildlife Service, Research Laboratory, IRL-Newtownmountkennedy, Co. Wicklow, Republic of Ireland

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Summary. The karyotypes of 46 animals from a population of *Cervus elaphus, Cervus nippon,* and hybrids *Cervus elaphus* × *Cervus nippon* were studied using G- and C-banding techniques. It was found that four chromosome pairs known from the karyotype of *Cervus elaphus* are involved in two systems of Robertsonian translocations. Pedigree analysis supports the hypothesis of a simple Mendelian inheritance of each translocation system. With respect to these translocation systems, the population shows no significant deviation from the corresponding Hardy-Weinberg-structure. Thus, hybridization events can be accepted as a major factor in karyotype evolution of deer.

Key words: Cervidae – Karyotype – Robertsonian translocation – Inheritance – Hybridization

Introduction

Intraspecific karyotype variation is a common phenomenon among the Cervidae. Most investigators explain this variation by the occurrence of Robertsonian translocations (Gustavsson and Sundt 1968; Gray 1971; Myake et al. 1982; Omura et al. 1983; Van Tuinen et al. 1983; Wang and Du 1983), but they provide no answer to the question as to how this variation is generated and maintained within a population. One hypothesis (Neitzel 1982) assumes that Robertsonian translocations are formed continuously by recent, selectively neutral chromosome mutations, with a tendency towards fixation due to a relatively high degree of inbreeding among the cervid social groups (Bützler 1974). Due to this tendency as well as to an assumed reproductive isolation effect, the translocations are supposed to have the capacity to initiate a speciation process by karyotypic divergence (Neitzel 1982). Another hypothesis (Bartoš and Žirovnicky 1981; Harrington 1979), supported by immunological (Bartoš and Žirovnicky 1981; Harrington 1985) and initial cytological (Harrington and Power 1985) studies, assumes that hybridization events between different taxa (e.g., species such as red deer and sika deer) cause Robertsonian polymorphisms in the hybrid populations. Moreover, an early morphological investigation on red × sika hybrids assumed that the sika deer from continental Asia, usually recognized as a subspecies of *Cervus nippon,* are hybrids between red deer and sika deer (Lowe and Gardiner 1975).

If these assumptions can be maintained, the concept that Robertsonian translocations promote speciation processes (Wilson et al. 1974; Neitzel 1982, 1987) needs some restriction, at least for the *Cervidae.* In particular, there is good evidence that Robertsonian translocations per se may be involved in speciation processes only if they cause reproductive failure, e.g., in the case of meiotic nondisjunction (King 1987).

The present paper addresses the questions of inheritance and stability of Robertsonian translocation chromosomes, as well as their distribution in a hybrid population. The results are interpreted with respect to the existing hypotheses on karyotype polymorphisms in the genus *Cervus.*

Materials and methods

Blood samples from 39 Irish red × sika hybrids, five Japanese sika deer (*Cervus nippon nippon*), and two Irish red deer were taken from the *Vena jugularis* into heparinized tubes after immobilization. In order to obtain the metaphase chromosomes, lymphocyte cultures were laid out and the metaphase chromosomes were studied by modified standard cytological techniques, as described previously in detail (Herzog 1985, 1988). The karyotypes of the red deer, skia deer, and red × sika hybrids have already been investigated by G- and C-banding techniques, in order to identify individual chromosome pairs and to homologize them with an existing standard idiogram of red deer (Herzog 1985, 1987 b). The use of banding techniques should enable the investigator to determine the origin of the translocation chromosomes in sika deer and the role of hybridization as well as of recent translocation events within a species in producing karyotype polymorphisms.

Proof of the mode of inheritance is lacking is nearly all previous papers on genetics and especially cytogenetics of wild animals. Nevertheless, this seems to be indispensable, and in the present study it has been accomplished by analyzing complete families (Table 2). Due to the relatively small number of related animals, the Exact Test (see Tate and Clelland 1957), leading to an exact level of significance, was the most appropriate statistical method for testing the hypothesis of inheritance. The observed genotypic structures were tested for deviation from the Hardy-Weinberg structure by the *G*-test (see Weber 1978). The arm length ratios of the meta- and submetacentric chromosomes were calculated from the average relative length of the p- and q-arms. The null hypothesis (equality of both averages in case of possibly unequal variances) was tested using the *t*-test (see Sachs 1984).

Results and discussion

The somatic, diploid chromosome number of all animals investigated varies between $2n = 64$, XX resp. XY and $2n = 68$, XX resp. XY), depending on the presence or absence of four submetacentric, pair-wise homologous autosomes (arm length ratios 0.85 and 0.71, respectively; see Table 1), while one metacentric chromosome pair is always detectable. The other autosomes are all telocentric and the autosomal nombre fondamental (N.F.$_A$) is 68 in all animals. The X-chromosomes are the biggest telocentrics in the set (see Figs. 1 and 2). The Y-chromosome is meta- to subtelocentric (Fig. 3), small, and therefore not unequivocally measurable. Its arm length ratio varies between 1.00 and 0.25.

The study of constitutive heterochromatin by C-banding shows patterns that are also very similar to those of red deer from Italy (Goldoni et al. 1984) and West Germany (Herzog 1985). No genetic variability of constitutive heterochromatin regions was observed, except that telocentric autosomes of the red deer occur as Robertsonian translocations in sika deer and in red × sika hybrids. Nevertheless, these translocation chromosomes show two distinct C-bands in the centromeric region, which means that no visible loss of constitutive heterochromatin took place after the translocation event. The weak C-bands of one metacentric chromosome pair, as well as the terminal C-(TC-)bands of the X-chromosome and the fully heterochromatic Y, are the same as in red deer (Goldoni et al. 1984; Herzog 1985).

Fig. 1. C-banded idiogram proposed for hybrids *Cervus elaphus × nippon*, using the typical karyotype of an F_1 generation male ($2n = 66$) as an example

Table 1. Arm lengths of chromosome pair 1 and translocation chromosomes 3/4 and 14/28. $\bar{x}$ = average arm length as a percentage of the diploid autosome set (revealed by measurements of 20 chromosomes each); s_x = standard deviation

Chromosome	Arm	$\bar{x}$	s_x	$\hat{t}$
1	p	1.06	0.12	
	q	1.09	0.14	0.779 NS
3/4	p	1.72	0.14	
	q	2.02	0.14	0.821*
14/28	p	1.05	0.10	
	q	1.46	0.13	11.156*

The comparison of the G-banded karyotypes with a standardized idiogram of the red deer (Herzog 1985, 1987 b) reveals a high degree of congruence. As G-banding (Fig. 2) shows, the metacentric pair detectable in all animals is chromosome pair 1 of the red deer. The two submetacentric pairs are translocations between red deer chromosomes 3 and 4, and 14 and 28. In the investigated population they form two independent systems of Robertsonian translocations, each with three phenotypes. A simple genetic hypothesis is that each of these two translocation systems segregates like one gene locus with two alleles. As Fig. 4 shows, two chromosomally homozygous types show both chromosomes of the involved pairs in the fused (two submetacentrics; symbol:

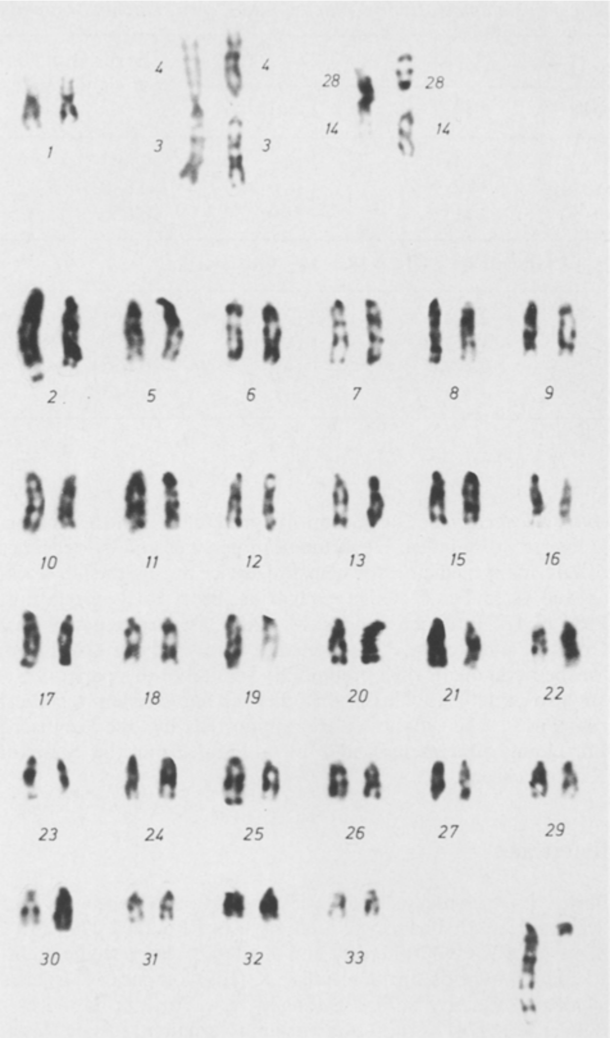

Fig. 2. G-banded idiogram proposed for hybrids *Cervus elaphus* × *nippon*, using the typical karyotype of an F_1 generation male (2n = 66) as an example

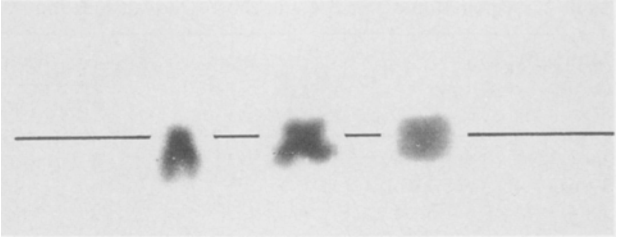

Fig. 3. Variation of the Y-chromosome in the animals studied (*Cervus elaphus*, *Cervus nippon*, and *Cervus elaphus* × *nippon*, C-banding)

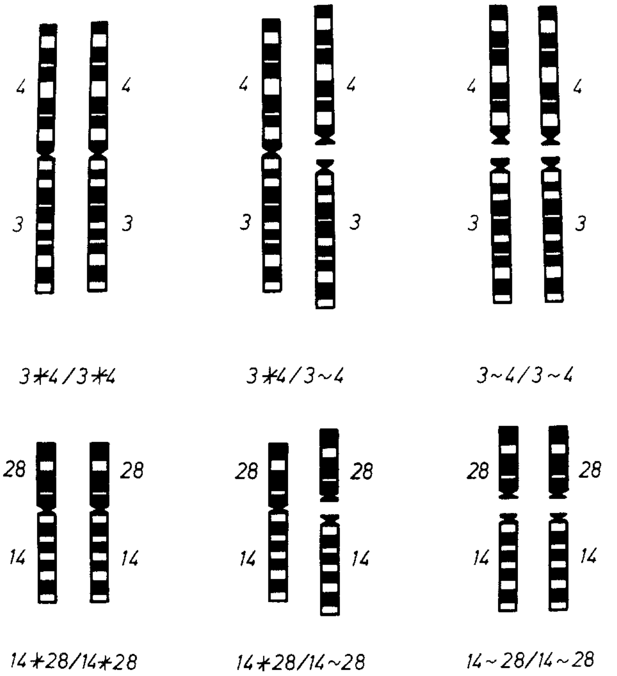

Fig. 4. Phenotypes of the Robertsonian translocation systems in *Cervus elaphus* × *nippon* as a diagram

3*4/3*4 and 14*28/14*28) and the nonfused (four telocentrics; symbol: 3~4/3~4 and 14~28/14~28) states respectively. The chromosomally heterozygous phenotype is characterized by one submetacentric translocation chromosome and, homologous to the arms of this submetacentric, two acrocentric chromosomes (symbol: 3*4/3~4 and 14*28/14~38). C-banding provides evidence that translocation chromosomes 3*4 and 14*28 are evolutionarily younger than chromosome 1, which is characterized by secondary loss of constitutive heterochromatin in the centromeric region and is assumed to have its origin in the Pliocene (Neitzel 1982, 1987). Genetic analysis of complete families (Table 2) supports this hypothesis.

All five of the deer classified as pure Japanese sika (*Cervus nippon nippon*) are chromosomally homozygous for both translocations, whereas both pure red deer investigated are chromosomally homozygous for the nonfused chromosomes. The two known F_1 hybrids exhibit the karyotype shown in Fig. 2, chromosomally heterozygous for the 3*4 and the 14*28 translocations.

All translocations found in the investigated population are stable and of the same 3*4 or 14*28 type. No evidence of meiotic or mitotic nondisjunction, especially in the chromosomally heterozygous types, has been found.

Finally, the results of the genetic analysis allow us to look at the genetic structures based on the distribution of

Table 2. Segregation of the 3/4 and the 14/28 translocation systems in *Cervus elaphus, Cervus nippon*, and *Cervus elaphus* × *nippon*

Mating-type	No. of pairs	No. of offspring[a]				Exact level of significance
		Total	3*4/3*4	3*4/3∼4	3∼4/3∼4	
3 * 4/3∼4 × 3 * 4/3∼4	3	8	2 (2)	4 (4)	2 (2)	0.897
3 * 4/3 * 4 × 3 * 4/3∼4	5	5	2 (2.5)	3 (2.5)	– (–)	0.688
3 * 4/3∼4 × 3∼4/3∼4	2	2	– (–)	2 (1)	– (1)	0.250
	Total		14 * 28/14 * 28	14 * 28/14∼28	14∼28/14∼28	
14 * 28/14∼28 × 14 * 28/14∼28	3	8	2 (2)	3 (4)	3 (2)	0.761
14 * 28/14∼28 × 14∼28/14∼28	2	2	– (–)	1 (1)	1 (1)	0.500
14 * 28/14 * 28 × 14 * 28/14∼28	4	4	2 (2)	2 (2)	– (–)	0.625
14 * 28/14 * 28 × 14 * 28/14 * 28	1	1	1 (1)	– (–)	– (–)	–

[a] Numbers in parentheses refer to expectations

Table 3. Genetic structures based on chromosomal diplo- and haplotypes with respect to the 3/4 and 14/28 translocation systems and comparison with the corresponding Hardy-Weinberg structures (HWS)

Diplo-/ haplotype	No. observed	HWS	Frequency observed	HWS	$\hat{G}$ (FG=1)
3 * 4/3 * 4	20	19.32	0.435	0.420	
3 * 4/3∼4	19	21.16	0.413	0.460	
3∼4/3∼4	7	5.52	0.152	0.120	0.618 NS
3 * 4	59	–	0.641	–	
3∼4	33	–	0.359	–	–
14 * 28/14 * 28	23	20.24	0.500	0.440	
14 * 28/14∼28	15	20.70	0.326	0.450	
14∼28/14∼28	8	5.06	0.174	0.110	3.664 NS
14 * 28	61	–	0.663	–	
14∼28	31	–	0.337	–	–

chromosomal haplo- and diplotypes within the populations (Table 3). A comparison of the observed genotype frequencies with the expected values under the assumption of a Hardy-Weinberg structure shows no significant deviation for either of the translocation systems studied.

Thus, the cytological as well as the genetic findings of the present study support the hybridization hypothesis (Harrington and Power 1985) of Robertsonian polymorphisms. Nevertheless, two of the previously described karyotypes exhibit other translocation types (Herzog 1985, 1987a) than the Irish population. They may be the product of hybridizations between other species of *Cervus*, a phenomenon that is well known in this genus (Powerscourt 1884; Benirschke 1967; Gray 1971). Thus, hybridization events can be accepted as a major factor in generating karyotype polymorphisms in sika deer, whereas recent Robertsonian translocations within one single species are assumed to have more qualitative than quantitative importance for the karyotype evolution in deer.

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